

# CYTOPLASMIC AND NUCLEAR MATURATION OF RABBIT OOCYTES *IN VITRO*

C. THIBAUT and Micheline GÉRARD

*Station de Physiologie animale, I. N. R. A.,  
73350 Jouy en Josas (France)*

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## SUMMARY

Rabbit oocytes from preovulatory follicles resume meiosis up to metaphase II in normal delay when cultured in TC 199 supplemented with rabbit and calf sera and chick embryo extract. They are fertilizable, but such oocytes are unable to assume transformation of the sperm head into a normal nucleus, the male pronucleus.

Rabbit preovulatory follicle can be maintained in healthy conditions for at least 24 hours, if gas pressure is increased to 5 to 10 bars/cm<sup>2</sup> during culture (air + 0.5 p. 100 CO<sub>2</sub>), and in the presence of gonadotropins.

In such follicles, oocytes remain in the dictyate stage when crude horse pituitary extract is added to the culture medium. However, if subphysiological doses of ovine LH or FSH (or better, FSH and LH) are present in the culture medium, meiosis resumes and complete cytoplasmic maturation occurs in all oocytes, as proved by normal fertilization and growth of the male pronucleus.

Thus, two steps may be considered in complete oocyte maturation :

1. The resumption of meiosis by rupture of the inhibitory effect of the granulosa layer. This is not basically dependent on gonadotropins.
2. On the contrary, passage of the male pronucleus growth factor, or its precursor, inside the oocyte is gonadotropic- follicular dependent.

These processes are both the necessary prerequisites to normal fertilization, and they can be activated in a pure *in vitro* system.

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The follicle constitutes a balanced physiological unit, the oocyte preventing luteinization of the granulosa and theca cells (NEKOLA and NALBANDOV, 1971), and the granulosa, the resumption of oocyte meiosis (FOOTE and THIBAUT, 1969).

This reciprocal control permits oocytes which have finished growth, to be stored for years in a dictyate stage in the ovary of female mammals.

Experimental detaching of the oocyte and its culture, or spontaneous detaching by granulosa picnosis, in the Graafian follicles in atresia, generally results in the resumption of meiosis up to metaphase II.

These oocytes can be fertilized and are also capable of division after parthenogenetic activation (THIBAUT, 1970, 1973). Is the only function of the ovulatory gonadotropin surge to insure the physiological and mechanical rupture of ties between the granulosa and the oocyte?

The progress of the fertilization of oocytes having thus finished nuclear maturation *in vitro* when analyzed shows that fertilization develops abnormally, and *in vitro* maturation of the oocytes outside their follicle is incomplete (THIBAUT and GÉRARD, 1971).

In the present study, we tried to determine the contribution of the follicle to complete oocyte maturation.

## MATERIAL AND METHODS

The oocytes or follicles used are taken from pubescent *New Zealand* doe rabbits usually nulliparous and in oestrus.

The largest follicles (6-12), considered as preovulatory follicles, are used.

### A. — Culture medium

The culture medium is composed of :

- TC 199-50 parts (Microbiological Associates)
- doe rabbit serum-15 parts (prepared by us)
- calf serum — 15 parts (Sorga)
- embryonic chick extract — 20 parts (prepared by us)

The gonadotropic hormones used are either sheep LH or FSH (C. N. R. S.), or unpurified horse pituitary extract (preparation we use in other circumstances to multiply the number of preovulatory follicles and obtain superovulation in the rabbit).

The steroid hormones (progesterone, 20 $\alpha$ -OH-progesterone 17 $\beta$ -oestradiol) are solubilized in alcohol.

All manipulations are done at 30°C, and the cultures at 37.5°C.

### B. — Obtention and culture of oocytes

Oocytes are aspirated with a glass pipette rinsed in a medium containing TC 199 and 10 p. 100 of doe rabbit serum, then cultivated in the medium described above.

Culture is done in tubes containing 1 ml of the medium. These tubes are placed in a glass dryer filled with a mixture of air + 5 p. 100 CO<sub>2</sub> which bubbles in the sterile water at the bottom of the dryer (THIBAUT and GÉRARD, 1971).

Time 0 is the moment when the oocytes are placed in final culture conditions. 15 to 25 minutes elapse between slaughter and time 0.

### C. — Obtention and culture of follicles

Under a binocular microscope the ovary is cut lengthwise into two unequal parts parallel to the ligament.

The narrowest part contiguous to the ligament is eliminated. It contains few or no follicles.

The other part is stretched crosswise and flattened by traction on the edges of the cut made when the ovary was sliced in two. The Graafian follicles then appear by transparency ; they are isolated from the interstitial tissue, other follicles and the ovarian epithelium by dilaceration with small brussels.

The follicles are then rinsed and cultured in Falcon dishes containing 0.8 ml of the final medium. 40 to 50 minutes elapse between slaughter and the time they are put in culture.

The dishes are placed in an air-tight metal box (fig. 5) which is filled with a mixture of air and a proportion of CO<sub>2</sub>, so that when gas pressure reaches 10 or 5 bars, depending on the experiments, the pH stays at 7.3-7.4 (about 0.5 p. 100 CO<sub>2</sub>).

When cultures are done at normal atmospheric pressure, the Falcon dishes are put in a glass dryer under 95 p. 100 air and 5 p. 100 CO<sub>2</sub>.

After culture from 8 to 24 hours in these conditions, the follicles are depressurized slowly for 10 to 20 minutes, then either fixed *in toto* or pierced, and their oocytes recovered and transferred into a fresh medium while waiting to be fertilized.

#### D. — Test of oocyte fertilization ability

The fertilization ability of oocytes cultured alone *in vitro* was tested by *in vitro* fertilization using the techniques ordinarily employed in the laboratory (THIBAUT and DAUZIER, 1961).

The fertilization ability of oocytes recovered after culture inside their follicles is tested by transferring these oocytes into the Fallopian tube of a doe rabbit mated 9 1/2 hours previously. The ovarian follicles in the ovary lying next to the Fallopian tube used, are cauterized at the time of transfer. The fertilization ability and evolution of pronuclei of the oocytes matured in their follicle *in vitro*, then transplanted into this Fallopian tube, may then be compared to that of control oocytes laid in the opposite Fallopian tube several minutes later.

#### E. — Cytological study of oocytes

The oocytes and follicles are fixed in Bouin-Hollande and the oocytes included in gelose, using the habitual techniques (THIBAUT, 1949). Serial sections are made at 10 μ, and the oocytes and follicles are examined after staining by hematoxyline eosin.

## RESULTS

### A. — Oocytes cultured *in vitro* independently of their follicle

We showed (THIBAUT and GERARD, 1971) that in the medium conditions described, all preovulatory oocytes undergo maturation *in vitro*. This maturation is chronologically normal because it occurs at exactly the same speeds as *in vivo* maturation after mating or HCG injection.

TABLE I

*Evolution of the sperm head from 3.5 hrs to 9 hrs after introducing capacited sperm in vitro*

*Évolution de la tête du spermatozoïde dans l'œuf de 3,5 à 9 heures après l'introduction du sperme capacité in vitro*

| Time after mixing (in h) | N° of eggs fertil. | No modification of sperm head |    | Membrane formation but no swelling |    | Small head pronucleus |    | Sub-normal male pronucleus |    | Cleaved eggs |    |
|--------------------------|--------------------|-------------------------------|----|------------------------------------|----|-----------------------|----|----------------------------|----|--------------|----|
|                          |                    | N°                            | %  | N°                                 | %  | N°                    | %  | N°                         | %  | N°           | %  |
| 3.30                     | 25                 | 23                            | 92 | 2                                  | 8  |                       |    |                            |    |              |    |
| 6.00                     | 12                 | 8                             | 66 | 3                                  | 25 |                       |    | 1                          | 9  |              |    |
| 7.30                     | 29                 | 9                             | 31 | 12                                 | 41 | 5                     | 17 | 3                          | 10 |              |    |
| 9.00                     | 7                  | 4                             | 57 | 2                                  | 30 |                       |    | 1                          | 13 |              |    |
| 15.00                    | 42                 | 3                             | 7  |                                    |    | 10                    | 24 | 8                          | 19 | 21           | 50 |

When these oocytes are placed *in vitro* in the presence of capacitated spermatozoa, the way the spermatozoid penetrates, the formation of the female pronucleus, the development of the spermaster, and the migration of the male and female nuclei towards each other in the center of the oocyte, occur normally, but the spermatozoon head does not change (fig. 6). There is no male pronucleus formation (THIBAUT and GERARD, 1970, 1971). Several hours after fertilization when the two pronuclei normally enter into contact in the center of the oocyte, only the junction of a female pronucleus with an unchanged sperm head may be seen.

Several hours later in some oocytes a nuclear membrane appears surrounding a practically unmodified sperm head. In only a few cases did a male pronucleus of subnormal size develop (table 1).

Absence of sperm head evolution, or late and always abnormal male pronucleus head formation, shows that during natural oocyte maturation in the *in vivo* follicle a substance appears in the cytoplasm which causes transformation of the sperm head in the male pronucleus. This substance is not present in the oocyte which undergoes *in vitro* maturation, although meiosis may be normal otherwise. We have decided to call this substance MPGF, male pronucleus growth factor.

#### B. — Origin of male pronucleus growth factor

In order to determine when this factor appears in the *in vivo* oocyte after the ovulatory surge, we recovered oocytes from preovulatory follicles 2, 3, 5 and 7 hours after mating or HCG injection.

TABLE 2

*Time required for the presence of the MPGF in the oocyte during in vivo maturation*

*Temps nécessaire à l'apparition du MPGF dans l'ovocyte  
au cours de la maturation in vivo*

| Time between<br>HCG, coitus<br>and culture<br>(h) | <i>In vitro</i><br>maturation<br>in hours | N° of<br>rabbits | N° of<br>oocytes | Fertilized eggs |    | Sperm head evolution     |               |
|---|---|------------------|------------------|-----------------|----|--------------------------|---------------|
|   |   |                  |                  | N°              | %  | No or<br>abnormal<br>(%) | Normal<br>(%) |
| 2   | 12.00                                     | 4                | 31               | 20              | 64 | 100                      | 0             |
| 3   | 12.00                                     | 2                | 15               | 8               | 53 | 100                      | 0             |
| 5   | 12.00                                     | 3                | 14               | 9               | 64 | 100                      | 0             |
| 7   | 5.00                                      | 3                | 25               | 11              | 44 | 20                       | 80            |

The oocytes are then cultured *in vitro* until nuclear maturation is finished, and then they are fertilized *in vitro*. Only those oocytes recovered at 7 hours after the ovulatory surge are capable of insuring the transformation of the spermatozoon into a normal male pronucleus (table 2).

MPGF thus appears late in the oocyte.

Changes in the Golgi apparatus, which are described in corona cells after the ovulatory surge (MORICARD, 1934, 1963), led us to think that these cells were responsible for the synthesis of this factor, but only in presence of gonadotropins. Therefore, we tried to cultivate oocytes from preovulatory follicles in presence of various hormones : FSH, LH, prolactin, 17  $\beta$ -oestradiol, progesterone or 20 $\alpha$ -OH progesterone. Only prolactin seems to have a slight effect favorizing the appearance of MPGF (table 3).

TABLE 3

*Influence of various hormones during in vitro maturation of the rabbit oocyte on the male pronucleus formation*  
*Influence de diverses hormones pendant la maturation in vitro de l'ovocyte de Lapine sur la formation du pronucleus mâle*

| Hormones                                     | N° of rabbits | N° of oocytes | Fertilized eggs |    | % digyny | Sperm head evolution (%) <sup>(1)</sup> |    |    |
|--|---------------|---------------|-----------------|----|----------|---|----|----|
|  |               |               | N°              | %  |          | 0                                       | *  | ** |
| FSH-ovine 2 $\mu$ g/ml                       | 5             | 48            | 21              | 44 | 9        | 62                                      | 25 | 13 |
| LH-ovine 2 $\mu$ g/ml                        | 5             | 38            | 24              | 61 | 30       | 95                                      | 5  | 0  |
| FSH + LH<br>2 $\mu$ g + 2 $\mu$ g/ml         | 4             | 33            | 30              | 90 | 40       | 93                                      | 7  | 0  |
| Prolactin 10 $\mu$ g/ml                      | 5             | 46            | 24              | 52 | 86       | 21                                      | 17 | 64 |
| FSH + prolactin<br>2 $\mu$ g + 10 $\mu$ g/ml | 4             | 25            | 19              | 76 | 50       | 69                                      | 12 | 19 |
| LH + prolactin<br>2 $\mu$ g + 10 $\mu$ g/ml  | 5             | 46            | 26              | 56 | 75       | 30                                      | 10 | 60 |
| Oestradiol 1 $\mu$ g/ml                      | 3             | 25            | 12              | 48 | 19       | 55                                      | 45 | 0  |
| Progesterone<br>10 $\mu$ g/ml                | 4             | 24            | 12              | 50 | 17       | 50                                      | 50 | 0  |
| 20 $\alpha$ -OH-Pg 10 $\mu$ g/ml             | 2             | 20            | 16              | 80 | 0        | 100                                     | 0  | 0  |

<sup>(1)</sup> 0 : No evolution.

\* : Late swelling or membrane formation.

\*\* : Small pronucleus.

### C. — Maturation in the follicle in vivo

The late presence of MPGF during *in vivo* maturation when the first polar body is about to be emitted, its absence in the oocyte when cultivated alone or even with its corona cells with or without gonadotropic or steroid hormones, indicate that neither passage into the oocyte cytoplasm of the germinative vesicle nuclear content nor production of corona cells is responsible for this factor ; it rather depends on the granulosa or theca cells.

This led us to inquire if it was possible to obtain MPGF synthesis by the *in vitro* follicle.

a) *Maintenance of the in vitro follicle without signs of degeneration.*

The study of oocyte intrafollicular maturation *in vitro*, under gonadotropic hormone stimulation, necessitates culture conditions in which the different layers — theca, granulosa, corona cells — will not present any sign of picnosis because the oocyte is able to spontaneously resume meiosis when the granulosa degenerates in the atresic follicles.

Noting BAKER and NEAL'S observation (1970) concerning the favorable role of pressure on mouse ovary follicles, we cultured preovulatory follicles under 10-bar pressure, with or without hormones. The follicles were examined 20-24 hours after being put in culture.

Table 4 clearly shows that the presence of gonadotropic hormones and a high pressure are equally necessary for procuring granulosa and/or corona cells with no sign of picnosis (fig. 1, 2, 3, 4).

TABLE 4

*Number of experiments with picnosed (P) or healthy (H) follicles under different conditions of culture*

*Nombre d'expériences avec des follicules normaux (H) ou picnotiques (P) selon les conditions de culture*

| Pressure : +<br>No pressure : 0 | Without hormones | With hormones              |         |           |          |
|---------------------------------|------------------|----------------------------|---------|-----------|----------|
|                                 |                  | HPE *                      | rat FSH | ovine FSH | ovine LH |
| 0                               | 4/4 P            | 1/1 P                      |         |           | 4/4 P    |
| +                               | 4/4 P            | 5/6 H<br>1/6 P<br>(slight) | 1/1 H   | 2/2 H     | 2/2 H    |

\* Horse pituitary extract. Extrait hypophysaire de cheval.

b) *Oocyte maturation.*

Two FSH and/or LH levels were used : 10 µg/ml or 1 µg/ml.

With each of the two hormones, and for the two levels used, 100 p. 100 oocytes finished meiosis (table 5).

Eight hours after the beginning of culture, the first polar body is almost formed. *In vitro* maturation thus seems to require about two hours longer than *in vivo* maturation. This may be due to the fact that *in vitro* the hormone must be diffused through the theca, while *in vivo* it is carried by the circulation to the granulosa basal membrane.

Eleven hours after beginning of culture, the second metaphase maturation spindle is formed.

Thirteen hours later, or 24 hours after beginning of culture, the oocytes remain in the same state and the cells of the various follicle layers are healthy.

TABLE 5

*Meiotic division induced by gonadotropins in follicular oocytes*  
*Induction de la méiose par les gonadotropines dans les ovocytes folliculaires*

| Duration of culture (hours) | N° of follicles | Responses to       |                                      |                     |                        |                  |                      |
|-----------------------------|-----------------|--------------------|--------------------------------------|---------------------|------------------------|------------------|----------------------|
|                             |                 | FSH<br>10 µg/ml    | FSH<br>1 µg                          | LH<br>10 µg         | FSH + LH<br>10 + 10 µg | LH<br>1 µg       | FSH + LH<br>1 + 1 µg |
| 24                          | 3               | 3 M <sub>2</sub> * | 6 M <sub>2</sub><br>5 M <sub>2</sub> | 6 M <sub>2</sub>    |                        |                  |                      |
|                             | 6               |                    |                                      |                     |                        |                  |                      |
|                             | 5               |                    |                                      |                     |                        |                  |                      |
|                             | 6               |                    |                                      |                     |                        |                  |                      |
| 24                          | 4               |                    |                                      |                     | 4 M <sub>2</sub>       |                  |                      |
| 24                          | 5               |                    |                                      |                     |                        | 5 M <sub>2</sub> |                      |
| 24                          | 4               |                    |                                      |                     |                        |                  | 4 M <sub>2</sub>     |
| 11                          | 3               | 3 M <sub>2</sub>   |                                      | 3 T <sub>1</sub> ** |                        |                  |                      |
|                             | 3               |                    |                                      |                     |                        |                  |                      |
| 8                           | 3               | 3 T <sub>1</sub>   |                                      | 3 M <sub>1</sub>    |                        |                  |                      |
|                             | 3               |                    |                                      |                     |                        |                  |                      |

\* M<sub>2</sub> : Metaphase II.

\*\* T<sub>1</sub> : Telophase I.

The follicle rarely ruptures, but it may appear to elongate, thus expressing a localized thinning of the wall.

*In vivo*, 24 hours after the LH peak, or 13 hours after ovulation, follicle luteinization is already quite advanced. 24 hours after gonadotropic hormone contact, in our *in vitro* experiments, follicle luteinization only occurs rarely. This may be explained by persistence of the inhibitory effect of the oocyte present in the follicle on granulosa cell luteinization (EL FOULY *et al.*, 1970 ; NALBANDOV, 1970).

Sometimes partial luteinization was observed with disappearance of the basal membrane and the granulosa invaded by theca cells already having a luteal aspect.

### c) Presence of MPGF.

MPGF is found in the oocyte when it is cultured inside the follicle for 12 hours in presence of FSH and/or LH at a dose of 10 µg/ml or 1 µg/ml. Soon after fertilization, the sperm head swells, and the male pronucleus forms a little while before the female pronucleus, as in control oocytes naturally ovulated and present in the opposite Fallopian tube. The process of fertilization is exactly the same in oocytes matured *in vitro* in their follicle as in control oocytes (fig. 7, 8 ; table 6). Nuclear and cytoplasmic oocyte maturation thus occurs completely *in vitro*, but inside the follicle and in presence of FSH or LH.

TABLE 6

*Fertilization of oocytes matured inside their follicles in presence of gonadotropins*  
*Fécondation des ovocytes maturés dans leurs follicules en présence de gonadotropines*

| Hormones<br>1 µg/ml | Oocytes matured in their follicles |            |                          | Control eggs           |                |
|---------------------|------------------------------------|------------|--------------------------|------------------------|----------------|
|                     | Number                             | Fertilized | Stage of fertilization * | Stage of fertilization |                |
| FSH {               | 5 bars {                           | 9          | 7                        | Stage 3                | Stage 3        |
|                     |                                    | 7          | 1                        | Stage 2                | Stage 2        |
|                     | 10 bars {                          | 6          | 3                        | 1 stage 2              | —              |
|                     |                                    |            |                          | 2 stage 3              |                |
| Total               | 22                                 | 11         |                          |                        |                |
| LH {                | 5 bars {                           | 6          | 5                        | 4 stage 4              | Stages 4 and 5 |
|                     |                                    |            |                          | 1 stage 2              |                |
|                     | 10 bars {                          | 10         | 5                        | 2 stage 4              | Stages 4 and 5 |
|                     |                                    |            |                          | 2 stage 3              |                |
| Total               | 16                                 | 10         | 1 stage 2                |                        |                |
| FSH + LH {          | 10 bars {                          | 9          | 9                        | Stage 4                | Stage 4        |
|                     |                                    | 6          | 6                        | Stage 3                | Stage 3        |
|                     | Total                              | 15         | 15                       |                        |                |

\* Stages according to THIBAUT, 1967.

## DISCUSSION AND CONCLUSIONS

Final nuclear and cytoplasmic maturation of the oocyte in mammals is insured by two different mechanisms.

The oocyte outside the follicle, with no gonadotropic hormone, in defined culture conditions, resumes meiosis spontaneously, and oocytes from preovulatory follicles attain the second metaphase of maturation exactly as *in vivo*.

The resumption of meiosis does not depend on steroids synthesized under gonadotropic effect, as in amphibians or fish, because as soon as oocytes are put in culture, the dictyate nucleus changes, metaphase I being formed at hour 4 after beginning of culture. Moreover, the fluid of mature rabbit follicles contains almost all the sex steroids, and LH only stimulates their synthesis (MILLS and SAVARD, 1972). We have proved that rupture of the inhibitory effect of the granulosa explains this rapid resumption of *in vitro* meiosis (FOOTE and THIBAUT, 1969).

We have also shown *in vitro* that FSH and LH electively act on corona

cells by causing their dissociation (THIBAUT, 1970), so that *in vivo* the oocyte is very quickly disconnected physically from the granulosa. This situation is very similar to that of the *in vivo* oocyte after ovulatory surge.

If the follicle is maintained in perfect *in vitro* survival without adequate gonadotropins, the oocyte remains in the dictyate stage (cow, sow : FOOTE and THIBAUT, 1969 ; ewe, woman, rabbit : FOOTE, MATLEY, TIBBITTS and THIBAUT, 1973 ; rat : TSAFRIRI *et al.*, 1972). On the other hand, meiosis is complete inside the cultured follicle in presence of sheep FSH and LH, as TSAFRIRI *et al.* (1972) have shown with rat follicles.

Cytoplasmic maturation can only occur inside the follicle, either *in vivo* or *in vitro*, and in presence of gonadotropins. The follicle furnishes the oocyte with the male pronucleus growth factor (MPGF) very late (hour 5-7). Since at that time cellular ties between oocyte and granulosa no longer exist, it is probable that, MPGF, or its precursor, is passed by means of the follicular fluid. If this is true this factor should be found in the follicular fluid.

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### RÉSUMÉ

#### MATURATION NUCLÉAIRE ET CYTOPLASMIQUE DE L'OVOCYTE DE LAPINE *IN VITRO*

La reprise de la méiose a été souvent observée dans les ovocytes de nombreux mammifères prélevés dans des follicules de de Graaf et cultivés *in vitro*, mais tous les ovocytes n'atteignent pas la métaphase II et leur aptitude à être fécondés et à se développer n'a pas été éprouvée.

Tous les ovocytes de Lapine prélevés dans des follicules préovulatoires de femelle en œstrus et cultivés dans un milieu contenant du TC 199, du sérum de Veau et de Lapin et de l'extrait embryonnaire de Poulet, achèvent leur méiose jusqu'à la métaphase II dans un délai identique à celui observé *in vivo* après coït ou induction de l'ovulation par HCG. Ces ovocytes sont fécondables mais ne permettent pas la transformation de la tête spermatique en un pronucleus mâle.

Les follicules préovulatoires peuvent être cultivés pendant au moins 24 heures sans picnose dans aucune des assises cellulaires du follicule ni dans les cellules périovocytaires à condition de maintenir l'atmosphère d'air et de CO<sub>2</sub> à une pression comprise entre 5 et 10 bars/cm<sup>2</sup> et en présence de gonadotropines.

Dans ces follicules, les ovocytes demeurent à l'état dictyé en présence d'extrait hypophysaire de cheval.

Si des quantités subphysiologiques de LH et/ou de FSH ovines sont ajoutées au milieu, la méiose reprend jusqu'à la métaphase II dans tous les ovocytes.

De plus, la maturation cytoplasmique complète se produit, comme le prouve le développement normal du pronucleus mâle si ces ovocytes sont fécondés. Les différents stades de la fécondation sont rigoureusement semblables à ceux des œufs témoins maturés et fécondés *in vivo*.

Ainsi, on doit considérer deux étapes dans la maturation finale de l'ovocyte avant l'ovulation :

1<sup>o</sup> La maturation nucléaire qui s'étend de la rupture du noyau dictyé à la métaphase II. Cette maturation est rendue possible par la rupture des liens cellulaires qui permettent à la granulosa d'exercer son action inhibitrice sur l'ovocyte. Cette étape ne dépend pas fondamentalement des gonadotropines puisqu'elle peut être réalisée par séparation expérimentale de la granulosa et de l'ovocyte sans aucun apport hormonal.

2<sup>o</sup> Au contraire, la maturation cytoplasmique qui correspond à la présence dans l'ovocyte de facteurs nécessaires à la croissance du pronucleus mâle (MPGF) implique l'action des gonadotropines sur les cellules du follicule lui-même et probablement sur la granulosa. Le passage de ce facteur ou de son précurseur dans l'ovocyte se fait par le liquide folliculaire.

Ces deux étapes de la maturation finale de l'ovocyte, préparatoires à la fécondation, peuvent être réalisés complètement *in vitro*.

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PLATES

## PLANCHE I

FIG. 1. — Follicule préovulatoire de Lapine cultivé 24 heures en présence d'extrait hypophysaire de Cheval à la pression atmosphérique (air + 5 p. 100 CO<sub>2</sub>). Remarquer les zones importantes de picnose dans la granulosa (P).

FIG. 2. — Culture sans hormones hypophysaires sous une pression de 10 bars (air + 0,5 p. 100 CO<sub>2</sub>). Petites zones de picnose. La méiose a repris jusqu'à la métaphase I dans l'ovocyte.

FIG. 3. — Culture en présence d'extrait hypophysaire de Cheval et sous une pression de 10 bars. Aucune picnose n'est visible. L'ovocyte est toujours au stade dictyé.

FIG. 4. — Culture en présence de 1 µg/ml de LH ovine et sous une pression de 10 bars. Toutes les cellules sont saines et le premier globule polaire s'est formé (visible à la gauche de l'ovocyte). On voit également dans l'ovocyte les chromosomes de la métaphase II. Ils ont été séparés en trois groupes par le rasoir.

## PLATE I

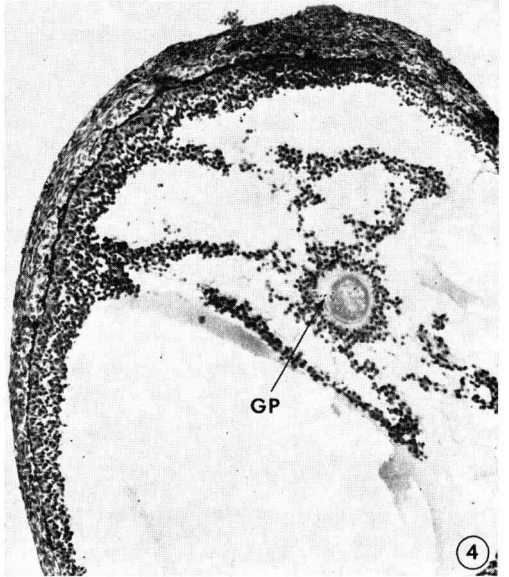
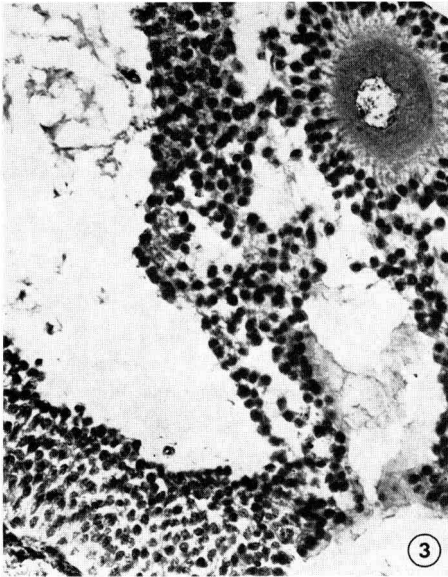
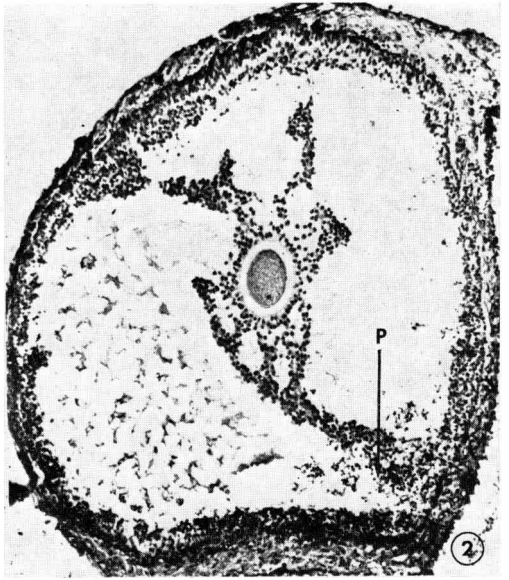
FIG. 1. — Rabbit preovulatory follicle cultured for 24 hours in presence of horse pituitary extract at normal atmospheric pressure (air + 5 p. 100 CO<sub>2</sub>). Note large areas of completely picnotic granulosa cells (P).

FIG. 2. — Culture without pituitary hormones under 10 bars of pressure (air + 0.5 p. 100 CO<sub>2</sub>). Small areas of picnosis (P). Oocyte has resumed meiotic division up to metaphase I.

FIG. 3. — Culture in presence of horse pituitary extract and under 10 bars of pressure. No picnosis was visible in any layers of the follicle. Oocyte has remained in the dictyate stage.

FIG. 4. — Culture in presence of 1 µg of ovine LH/ml and under 10 bars of pressure. All the cells of the follicle are healthy and first polar body is clearly visible on the left side of the oocyte (PB). Metaphase II, chromosomes are also visible in the oocyte. They are scattered in three groups by sectioning.

PLATE I



## PLATE II

### FIG. 5

Details of metal box used for culture under pressure

### FIG. 6

*In vitro* matured oocyte outside its follicle and *in vitro* fertilized. Note the absence of sperm head evolution.

### FIG. 7

*In vitro* matured follicular oocyte then fertilized *in vivo* in the right fallopian tube of a rabbit mated 9 hrs before transfert. Evolution of male pronucleus is absolutely normal.

### FIG. 8

Normally ovulated and fertilized egg from the left fallopian tube of the same rabbit.

## PLANCHE II

### FIG. 5

Détails du caisson métallique utilisé pour les cultures sous pression.

### FIG. 6

Ovocyte mûré *in vitro* hors de son follicule et fécondé *in vitro*. Remarquer l'absence d'évolution de la tête spermatique.

### FIG. 7

Ovocyte mûré *in vitro* dans son follicule, sous pression et en présence de FSH et LH, fécondé *in vivo* dans l'oviducte droit d'une lapine accouplée 9 heures avant le transfert. L'évolution du pronucleus mâle est absolument normale.

### FIG. 8

Œuf témoin de l'oviducte gauche de la même lapine, ovulé normalement.

PLATE II

